which result is more convincing. Altered miRNA expression has been clearly linked to liver cancer, but the molecular mechanisms by which miRNA modulates hepatocarcinogenesis are still unknown. The reasons for their controversial study results are complex; we think lack of consideration of risk factors for chronic HBV-related HCC in these studies may be one of the key reasons.

In summary, well designed and systematic studies are still needed to clarify the molecular mechanism that leads to deregulation of miRNA expression in HBV-associated multistep hepatocarcinogenesis. Incorporating HBV DNA level, sex, age, cigarette smoking, alcohol consumption, chemical carcinogens, hormonal factors, and genetic susceptibility when investigating the relationship between deregulation of miRNA expression and HCC may be helpful in resolving the controversies.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: "Deregulation of microRNAs expression occurs in stages of multistep hepatocarcinogenesis: Why is it different?"

To the Editor:

Yin *et al.* drew attention to the differences between the findings on miR-221, miR-21 and miR-122 deregulation in hepatocarcinogenesis reported in our recent study [1] and others [2–4]. Yin *et al.* further speculated that the inconsistence of the findings among these reports might be due to different risk factors for HCC and called for further investigations on this subject.

In our article, we reported findings on the expression levels of seven cancer-related miRNAs in a series of chronic hepatitis/cirrhotic livers, dysplastic nodules (DNs) and small HCC samples. We found that miR-145, miR-199b and miR-224 were significantly deregulated in early stages of hepatocarcinogenesis (DN and small HCCs) when compared to the corresponding non-tumorous livers. The evidence for miR-21, miR-221, miR-10b and miR-122 deregulation was less clear and the data did not reach the stringent statistical threshold employed for that study (p <0.001 for one-way ANOVA and post tests with Bonferroni correction for multiple comparisons). However, we have to emphasize that our analysis mainly focused on the early stages of hepatocarcinogenesis and whether a miRNA was deregulated in DNs and small HCCs. Although our findings failed to demonstrate a statistically significant deregulation of miR-21, miR-221, miR-10b, and miR-122 in early stages of hepatocarcinogenesis, our findings in no way excluded the possibility of deregulation of these miRNAs in later stages of HCC. In fact, we found that miR-221 and miR-122 were significantly deregulated in early stages of HCCs when compared to the non-tumorous liver samples (p <0.001, post test with Bonferroni correction, Supplementary Fig. 1). An increasing trend was also observed with miR-21 when small HCCs were compared with the non-tumorous livers. These findings were actually in line with those of the previous reports [2–4].

In principle, we agree with Yin *et al.* that risk factors may affect the miRNA expression pattern in HCCs. In our study, we focused on the miRNA deregulation in HBV-associated HCC [1]. Indeed, the HCC samples used in the above-mentioned studies had substantially different etiological backgrounds when compared to our sample cohort. Kutay *et al.* mainly focused on premalignant nodules and HCC samples obtained from foliate and methyl-deficient (FMD) diet-fed Fisher 334 rats, while the risk factors of the three human HCC samples in their study were not described [3]. In the study of Pineau *et al.* the HCC samples

were mainly (>75%) HCV-associated [4], and the risk factor was different from that of our samples. For the 19 HCC samples used by Connolly *et al.*, nine were HBsAg positive but the HBV status was not available in the remaining 10 cases. In addition, these HCC samples were collected from Qidong area of Jiangsu Province of China, where aflatoxin B1 is a common risk factor of HCC [2]. The diversity of the etiological background of the samples, together with the various experimental approaches involved, very much hinders direct comparison of data among the different studies. For this reason, experimentally unified and well controlled studies on large sample cohorts are required to investigate the effects of different etiological factors on miRNA deregulation in human HCCs.

Conflict of interest

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Is a liver biopsy necessary in alcoholic hepatitis?

To the Editor:

The paper by Mookerjee *et al.* allowed a fascinating insight into the prognostic relevance of the systemic inflammatory response (SIRS) in decompensated alcoholic liver disease (ALD), especially the presence of alcoholic steatohepatitis (ASH) [1]. However, the paper makes some assertions which require further discussion.

The main assertion is that a liver biopsy is essential to determine the diagnosis and prognosis in patients with ASH. Not only is this contrary to recently published guidelines [2], there are practical issues in obtaining and interpreting transjugular liver biopsies. Few liver centres in the United Kingdom can readily provide such a service. In 2009/10 there were 14,700 hospital admissions in England alone for alcoholic liver disease: 5700 with "alcoholic cirrhosis" and 1600 with "alcoholic hepatitis" [3]. Mookerjee *et al.* describe biopsies in 71 patients over a 3-year period. The provision of transjugular liver biopsies throughout the rest of the United Kingdom and Europe for the thousands of additional ALD patients is unrealistic. A management strategy based upon an impractical level of investigation will not benefit most patients with ALD. We must ensure that we can assess and effectively manage these patients in all hospitals and not solely in limited specialist centres.

With regards to the need for biopsy for diagnosis of alcoholic hepatitis, the figure of 70–80% accuracy of a clinical diagnosis subsequently confirmed by biopsy is often quoted [2]. However, the most consistent difference between patients with alcoholic hepatitis and other patients with decompensated ALD is the degree of hyperbilirubinaemia [4,5]. A recent review of all the randomised controlled trials of treatments for alcoholic hepatitis determined the accuracy of the clinical criteria used relative to subsequent histological confirmation. If only those studies, which used a minimum level of bilirubin (ranging from 80 to 100 μmol/L) or whose patient population had a lower limit of bilirubin greater than 80 μmol/L, were considered, the accuracy of a clinical diagnosis rose to 96% [6]. This is very similar to a group of patients with ALD presenting with Acute-on-Chronic Liver Failure (ACLF) which has recent onset of hyperbilirubinaemia (>85 μmol/L) as a major tenant of its definition [7]. In this group of patients again, 96% had features of ASH. In the paper by Mookerjee, the criteria for a clinical diagnosis of ASH appear to have been decompensated ALD with the presence of SIRS without a minimum value of bilirubin stipulated. "Progressive jaundice" was just one of three criteria used to define the patient group studied. Furthermore, no information was provided regarding duration of liver disease at time of biopsy. Previous experience indicates that the accuracy of clinical diagnosis of alcoholic hepatitis is highest if the biopsy is performed within 4 weeks of initial presentation [8].

Whilst Maddrey's Discriminant Function (DF) has been a landmark advance in the assessment of alcoholic hepatitis, its accuracy is in question not least of all because it relies upon the measured prolongation of the prothrombin time, which is prone to significant laboratory variation. Additionally, in the paper by Mookerjee, the DF was not found to be predictive of 28-day outcome. More recently, the MELD score and the Glasgow Alcoholic Hepatitis Score (GAHS) have been shown to identify poor outcome from alcoholic hepatitis [2,9]. These have similar AUC values to those described for the histological ASH grade described in the paper by Mookerjee *et al.* The paper did not demonstrate that their histological data were independently predictive of survival or added significantly to the predictive value of these scores. The development of a reproducible histological score for alcoholic hepatitis is to be applauded. However, even in this specialist centre there was a delay of up to 7 days before the biopsies were obtained. With the additional time for preparation and interpretation of the specimens, the delay in obtaining a prognosis from histological criteria is significant and the opportunity to intervene early in those patients who may benefit may be lost. However, clinical scores such as the GAHS, readily calculable on the day of admission and over subsequent days, are not only accurate